DDT: Participation in Ultraviolet-Detectable, Charge-Transfer Complexation

Abstract. *The chlorophenyl groups of DDT and several of its metabolites are capable of participating in a charge-transfer interaction with tetracyanoethylene detectable in the ultraviolet region of the spectrum. In addition, during a change of state DDT undergoes ultraviolet spectral alterations that closely resemble those previously claimed to support the hypothesis suggesting charge-transfer interaction between this pesticide and a component of insect nerve tissue. The pesticide DDT possesses structural characteristics that would permit it to participate in several types of molecular association.*

A consideration of the toxicological and pharmacological characteristics of the aromatic chlorinated hydrocarbon pesticides indicates that the effectiveness of DDT (*1*) generally equals or exceeds that of many DDT metabolites in eliciting symptoms of neurotoxicity (*2*), in inhibiting membranal adenosine triphosphatases (*3*), in inducing uterine protein synthesis (*4*), and in inducing microsomal oxidase activity (*5*). Initiation of each of these types of biological responsiveness is generally assumed to result from physical interaction (binding) of DDT with one or more responsive biological molecules. Although several general models have been proposed to account for the neurotoxicity of DDT (*6*), the report that DDT formed a charge-transfer complex with nerve axon protein detectable in the ultraviolet region (*7, 8*) suggested that the pesticide may effect neurotoxicity as a result of binding to one or more uniquely responsive membranal components. In this report we demonstrate that the hypothetical involvement of DDT in ultraviolet-detectable, charge-transfer complexation is supported by results from experiments in which a conventional electron acceptor is used to interact with the pesticide.

The spectrum reported by Matsumura and O'Brien (*8*) to represent a charge-transfer complex involving DDT and an uncharacterized component of cockroach nerve cord appeared somewhat unusual. The reported spectrum obviously did not represent partially complexed DDT, as might be expected from the interaction of two different molecular species in a third solution continuum. In order for the reported spectrum of the DDT complex to have the implied significance, it was necessary that all of the DDT exist as a molecular complex—perhaps, resembling the intercalated benzpyrene-DNA complex (*9*).

We have observed that the ultraviolet spectral phenomena that accompany the change of state which occurs when an alcohol-water *solution* of DDT is converted to an alcohol-water *suspension* revealed a strikingly significant similarity to the ultraviolet spectral phenomena that were suggested (*8*) to represent charge-transfer complexation of DDT with a nerve component.

Spectra were collected at 27°C in standard silica cells (light path, 1 cm) with a recording spectrophotometer (Beckman DK 2A). Reagent grade chemicals were used.

The ultraviolet spectra of DDT are qualitatively similar in hexane, isoamyl alcohol, isobutanol, 2-propanol, or 30 percent 2-propanol in water. The absorption maxima, λ_{max}, occur at 220 and 237 nm, with a shoulder at 234 nm.

Although $3 \times 10^{-5}M$ DDT in 30 percent 2-propanol in water formed a stable solution, $2 \times 10^{-5}M$ DDT in 20 percent 2-propanol in water underwent time-dependent interactions which resulted in the formation of a visibly detectable suspension after the "solution" had been allowed to stand for 60 minutes at room temperature.

The ultraviolet spectrum of a freshly prepared "solution" of DDT in 20 percent 2-propanol in water indicated a single absorption maximum at 240 nm in conjunction with a shoulder extending above 300 nm. This shoulder provided preliminary evidence of light scattering. The absorption at 240 nm decreased with time, and a second band, with $\lambda_{max} = 248$ nm, became detectable (Fig. 1).

The initial absorbance (intensity), at 240 nm, of the $2 \times 10^{-5}M$ DDT "solution" in 20 percent 2-propanol in water was essentially the same as that of $2 \times 10^{-5}M$ DDT in 2-propanol at 237 nm (Fig. 1). A clear supernatant, resulting from centrifugation of a day-old DDT suspension at 30,000*g* for 20 minutes at 25°C, showed no absorbance in the ultraviolet region.

The ultraviolet absorbance changes that were observed upon examining freshly prepared $2 \times 10^{-5}M$ DDT "solution" in 20 percent 2-propanol in water were assumed to represent evidence of self-association of DDT. It is conceivable that the apparent bathochromic shifts in the ultraviolet spectrum could represent intermolecular charge-transfer complexation of DDT with DDT; how-

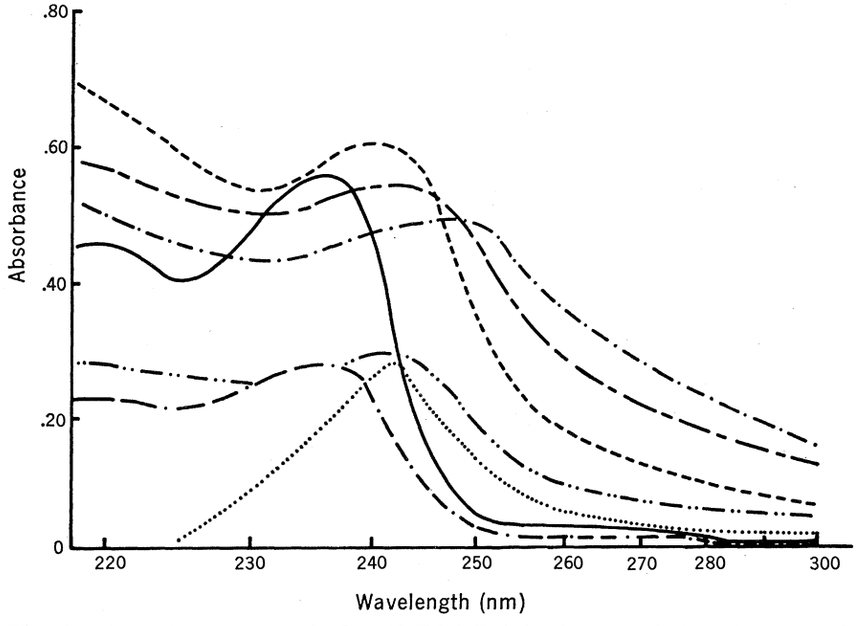

Fig. 1. Absorption spectra of: $2 \times 10^{-5}M$ DDT in 2-propanol (——); $2 \times 10^{-5}M$ DDT in 20 percent 2-propanol in water 1 minute after preparation (- - - -), 5 minutes after preparation (— -), and 30 minutes after preparation (— •); $1 \times 10^{-5}M$ DDT in 2-propanol (- - •); and $1 \times 10^{-5}M$ DDT in 10 percent 2-propanol in water with a 10 percent solution of 2-propanol in water as reference solution (— • •) and with 2-propanol as reference solution (• • • • •).

ever, such phenomena are, at best, poorly understood.

As a technique variation, pure 2-propanol was used as the reference solution during collection of the spectrum of $1 \times 10^{-5}M$ DDT "solution" in 10 percent 2-propanol in water (Fig. 1). The spectrum obtained in this manner, immediately upon preparation of the DDT suspension, is qualitatively the same as that reported to represent a charge-transfer complex involving DDT and some unknown component of cockroach nerve cord (*8*).

A $1 \times 10^{-4}M$ solution of DDT in 2-propanol was added to a solution of bovine serum albumin (500 μg ml^{-1}) or to a suspension of calf brain microsomal ($Na^+ + K^+$) activated adenosine triphosphatase (50 μg of protein per milliliter) (*10*) to yield a final DDT concentration of $1 \times 10^{-5}M$. In both types of protein-DDT mixtures difference spectra were qualitatively indistinguishable from the spectra of $10^{-5}M$ DDT suspensions in 10 to 20 percent alcohol in water (Fig. 1). These protein-DDT preparations also reflected temporal ultraviolet-absorption variations similar to those obtained with alcohol-water mixtures containing DDT.

Several studies with model systems may be interpreted to indicate that the trichloromethyl group (*11; 12*, pp. 52–55), the benzhydryl system (*13*), and the chlorophenyl groups of DDT possess characteristics that are compatible with their possible involvement in charge-transfer complexation or other types of molecular complexation, or both. We have obtained direct ultraviolet-visible spectral evidence in support of the hypothesis that the *p*-chlorophenyl groups of DDT can participate in charge-transfer interaction with tetracyanoethylene (TCNE). Interaction of DDT with iodine was also investigated.

Visible bands in the spectra of iodine complexes with electron donors—benzene, toluene, trimethylbenzene—shift toward the ultraviolet as the donor becomes increasingly more electron-releasing in character (*12*, p. 16). These shifts are generally assumed to result from the transfer of an electron from the donor to an antibonding orbital of the halogen in the excited state. It had earlier been observed (*14*) that benzene is only a slightly better electron donor than monochlorobenzene: that is, λ_{max} for I_2 in cyclohexane is at 520 nm; λ_{max} for I_2 in chlorobenzene is at 505 nm; λ_{max} for I_2 in benzene is at 500 nm.

Charge-transfer spectra for solutions of TCNE complexes are particularly convenient to investigate as they extend into the visible region. Tetracyanoethylene in chloroform is colorless, whereas TCNE forms yellow complexes with chlorobenzene, benzene, and toluene. The TCNE charge-transfer band is much more intense with toluene than with benzene, and it is weakest with chlorobenzene. The λ_{max} of the TCNE-toluene band is at 402 nm, whereas the λ_{max} of the TCNE-benzene and TCNE-chlorobenzene bands occur at about 380 nm (*14*).

Although we have been unable to obtain evidence of charge-transfer complexation of DDT with iodine, TCNE forms yellow complexes with *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDMS, *p,p'*-DDA (*1*), and bis(*p*-chlorophenyl)-methane. The TCNE charge-transfer bands for DDT and DDD (Fig. 2), as well as that for DDMS, are essentially the same, and they simulate that for chlorobenzene upon correction for absorption overlap from the initial high concentrations of solutes.

The absorbance of the TCNE complexes with DDT, DDD, and DDMS was much lower than would be expected from equivalent concentrations of chlorobenzene. This observation suggested that, although the chlorophenyl substituents on DDT, DDD, or DDMS may enter into charge-transfer complexation with TCNE, the chlorophenyl groups of these compounds are weaker electron donors than chlorobenzene. Confirmation of this analysis was indicated by the four- to fivefold increase in the absorbance of the charge-transfer band of TCNE-bis(*p*-chlorophenyl)-methane relative to that of TCNE-DDT.

Substitution of one hydrogen on the methane carbon in bis(*p*-chlorophenyl)methane with a mono-, di-, or trichloromethyl group greatly reduced the capability of the *p*-chlorophenyl groups to act as electron donors. This apparent intramolecular electron-withdrawing effect is consistent with a model depicting a high electron density in the ostensibly aliphatic chlorine atoms of DDT, DDD, and DDMS. These properties of DDT are compatible with a model in which the trichloromethyl group would be capable of a significant intermolecular dipole-dipole or dipole–induced dipole interaction in a biological system, whereas the *p*-chlorophenyl groups could participate in intermolecular interactions (including charge-transfer) involving their aromatic π electron systems.

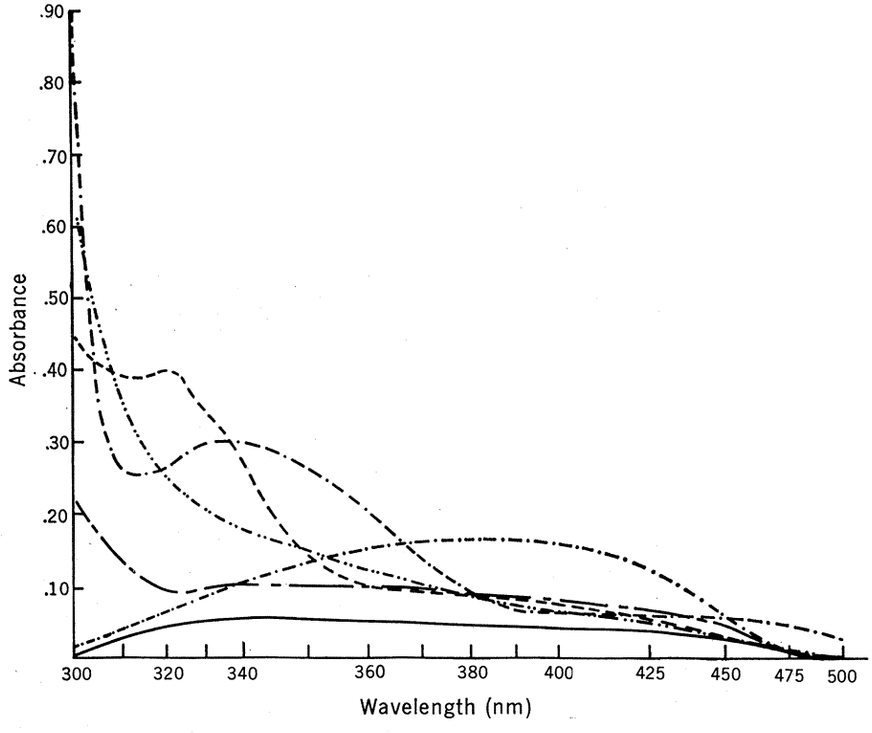

Fig. 2. Absorption spectra of charge-transfer complexes of chloroform solutions of $1.5 \times 10^{-3}M$ tetracyanoethylene with $0.4M$ chlorobenzene (— •); $0.4M$ *p,p'*-DDT (— -); $0.4M$ *o,p'*-DDT (- - - -); $0.1M$ *p,p'*-DDA (— • •); $0.2M$ *p,p'*-DDD (——); and $0.1M$ bis(*p*-chlorophenyl)methane (— — —•). The reference solution in each case was $1.5 \times 10^{-3}M$ tetracyanoethylene.

More precise characterization of possible DDT molecular association phenomena could be obtained from studies of solution behavior in which nuclear magnetic resonance spectrometry (*13, 15*) is used and from correlations of colligative properties with charge-transfer characteristics of appropriate molecular complexes of pesticides.

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References and Notes

1. Abbreviations are: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; DDMS, 1-chloro-2,2-bis(*p*-chlorophenyl)ethane; DDA, 1,1-bis(*p*-chlorophenyl)acetic acid.
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Silent Hemoglobin Alpha Genes in Apes: Potential Source of Thalassemia

Abstract. *Small quantities of unusual hemoglobins were found in 1 of 37 chimpanzees and 2 of 6 gorillas. In each genus these hemoglobins contain unique α chains that differ from the ordinary by eight to nine scattered amino acid changes. The unusual chains arise from a hitherto undetected* hemoglobin $^3\alpha$ *locus. No* $^3\alpha$ *products are found in most apes; accordingly,* $^3\alpha$ *is considered synthetically inactive in all but a few reversion mutants. Indirect evidence that the inactive* $^3\alpha$ *locus is juxtaposed to an active α locus together with the supposition that* $^3\alpha$ *exists in man provides a setting wherein thalassemia might be produced by nonhomologous recombination between two loci.*

Silent genes, that is, genetic loci without demonstrable products in most individuals of a species, have not been heretofore identified in higher organisms. In this report we provide reasons for believing that a silent locus, termed *hemoglobin* $^3\alpha$, exists in great apes and probably also in man. In most individuals $^3\alpha$ seems to be inactive and produces no evident product; however, in a few mutants the locus is active and produces an unusual α chain.

During an electrophoretic survey of adult hemoglobins from great apes, three exceptional animals were encountered. One of 37 (*1*) chimpanzees (*Pan troglodytes*) and 1 of 5 (*1*) unrelated lowland gorillas (*Gorilla gorilla gorilla*) exhibited not only hemoglobins A and A_2 but also small quantities (2.4 to 3.4 percent, Fig. 1 legend) of an unusual form of hemoglobin A and still smaller quantities (0.04 to 0.1 percent) of an unusual A_2. Both components differed from the usual by a net gain of about four electrostatic changes per hemoglobin molecule. Identical amounts of these unusual components were also found in the son of the variant gorilla (*2*). Electrophoresis of isolated (*3*) concentrates of the principal unusual components, designated hemoglobin Hyzoo in the chimpanzee and hemoglobin Wazoo (*4*) in the gorilla, are shown in Fig. 1 (*5*).

Parallel variation of both hemoglobin A ($\alpha_2\beta_2$) and A_2 ($\alpha_2\delta_2$) in all affected individuals suggested that an unusual α chain was present in these animals. This was corroborated by column chromatographic separation of constitutive hemoglobin chains (*6*). Chromatographic behavior and amino acid composition of β chains from both Hyzoo and Wazoo were identical to A-β. In contrast, the α chains of Hyzoo and Wazoo each showed net gains of about two electrostatic changes when compared with A-α from variant animals. After whole chain analysis, both variant α chains were distinctly unusual in the proportions of particular amino acids among the total of 141 residues present (*7*).

The differences between variant α and A-α sequences were further dissected through amino acid analysis of purified tryptic peptides (*7*). The net number of various residues realized from the sum of tryptic peptides exactly matched those obtained by whole chain analysis, thereby suggesting that characterization of variant chains is reasonably complete. A synopsis of differences is shown in Fig. 2. A remarkable feature—pivotal to our later interpretation—is the similarity between chimpanzee (*Pan*) Hyzoo-α and *Gorilla* Wazoo-α. These chains share a presumed constellation of eight scattered amino acid differences, outlined in Fig. 2, with respect to the A-α sequences characteristic of each genus.

The extent and diffuse distribution of differences shown in Fig. 2 make it most unlikely that either Hyzoo-α or Wazoo-α, let alone both, have arisen simply as allelic mutations at the locus for A-α. Detectable hemoglobin mutants differ from wild-type alleles, either through changes in one nucleotide or, in a few instances, through deletion of short runs of nucleotides in multiples of three (*8*). Aside from a few instances of within-locus recombinants between two separate nucleotide changes, multiple scattered changes are not found among uncommon variants. Multiple scattered differences may, however, develop between common alleles (*9*) when these have been maintained by natural selection for millions of generations. In this connection both Hyzoo and Wazoo are distinctly uncommon; nothing like them was detected in other surveys involving samples from substantial numbers of great apes (*10*). As persistently rare alleles at the locus for A-α these variants would, by Fisher's prediction (*11*), be lost long before they could accumulate step by step the pattern of change shown in Fig. 2. Accordingly, Hyzoo-α and Wazoo-α can only be regarded as the products of an α locus that is separate from the locus for A-α. It is likely that this additional α locus has a common ancestry in the two species, that is, it arose from a single gene duplication in some common ancestor of apes. Although six of the eight positions wherein Hyzoo-α and Wazoo-α are seemingly alike and different from A-α